Influence of Plant Protection Products on the Enzymatic Activity of Calcic Chernozem

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Abstract. The studies were carried out in Rostov region in order to assess the pesticides treatments effect on the enzymatic activity of calcic chernozem. The activities of catalase, invertase, phosphatase and urease were studied. The cultivated crop is chickpeas Donplaza variety. The experiment scheme included 8 variants: control; two variants of chemical plant protection, a biological plant protection system on unfertilized and fertilized backgrounds. The studied chernozem is classified as moderately enriched and rich in catalase, moderately enriched in invertase and phosphatase, and poor in urease. Catalase activity in the arable horizon varied in all variants within the range of 9.6–12.3 ml O_2/1 g/1 min, invertase activity – 30.4–36.8 mg glucose/1 g/24 h, phosphatase activity – 2.3–4.3 mg P_2O_5/10 g /1 h, and urease activity–4.5–8.1 mg N-NH_3/10 g/24 h. The treatment of soil and plants with chemical protection agents has a different effect on various enzymes, reducing the activity of catalase, invertase and stimulating the phosphatase activity. The treatment with biological plant protection agents led to stimulation of catalase activity. The invertase and urease activities were inhibited and the phosphatase activity remained at the same level.

Keywords: Chernozem · Enzymatic activity · Catalase · Invertase · Phosphatase · Urease · Pesticides

1 Introduction

The required element of modern crop cultivation technologies is the use of various plant protection products, which, in addition to a positive effect, also has negative consequences: soil and environmental pollution with pesticides, a decrease in biological activity and environmental stability of the soil.

Pesticides, getting into the soil, can accumulate in it, interact with soil microorganisms, and also enter adjacent environments such as groundwater, rivers, etc. The safety of the pesticides use can be assessed both on the basis of environmental and toxicological indicators, and on the basis of studying the indicators of the soil biological activity, in particular, the activity of soil enzymes [1, 2].

Soil enzymatic activity is a set of biochemical processes catalyzed by extracellular soil enzymes, most of which are fixed on soil particles or stabilized in soil solution.

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Soil enzymes are natural biocatalysts, which are high-molecular protein substances, the sources of which can be plants, microorganisms, algae, fungi, animals. Enzymes significantly accelerate biochemical reactions (tens and hundreds of times), and make it possible for these reactions to proceed at normal temperature. Soil enzymes catalyze specific processes of transformation of organic residues and humus involved in the biochemical cycles of carbon, nitrogen, phosphorus, and other elements [3, 4].

The level of soil enzymatic activity is determined not only by the biotic component of the soil, but also by its hydrothermal regime, agrochemical and physicochemical properties. Anthropogenic impact (mechanical processing, application of mineral and organic fertilizers, plant protection chemicals and growth stimulants) is also a powerful soil-forming factor in the soils of agrocenoses. Most of the preparations introduced into the soil have high physiological and chemical activity, therefore, even in small concentrations, they can change the level of soil enzymatic activity [5, 6]. Pesticides entering the soil can destroy soil enzymes, activate or inhibit their action, and also affect the sources (microorganisms and plants) that produce enzymes [2, 7].

The purpose of the work is to study the effect of pesticide treatments of chickpea crops on the enzymatic activity of ordinary carbonate chernozem under the conditions of the Azov agricultural zone of the Rostov region.

2 Materials and Methods

The experiments were laid in 2019–2021 on the experimental fields of the Federal Rostov Agrarian Research Center, which are located in the Aksai district of the Rostov region. The object of study is an ordinary calcareous heavy loamy chernozem, which is referred to as Calcic Chernozem (Loamic) [8]. Cultivated crop is chickpea of "Donplaza" variety (predecessor - winter wheat). The experiment was laid in triplicate on unfertilized and fertilized (N40P40K40) backgrounds (Table 1).

Soil samples were taken from the topsoil in April before treatment with protective agents and in June. On the experimental plots, agrotechnologies for chickpea cultivation recommended for the Azov zone of the Rostov region were used.

The herbicide Gezagard (500 g/l of promethrin), which belongs to the chemical class of triazines, was used in the variant of the chemical protection system 1. Depending on the amount applied to the soil, the herbicide retains its activity for 2–14 months. Due to their low solubility in water, triazines are retained in the upper soil layer and are subjected to the usual impact processes, such as decomposition under the action of light, absorption by soil colloids and plants, and, to a lesser extent, evaporation and leaching.

Herbicides based on triazines are the least toxic among other classes of chemical compounds; however, an increase in its dosage leads to a decrease in all groups of microorganisms [9]. This should be taken into account, since for soils with a heavy granulometric composition, which are the chernozems of the Northern Azov region, higher dosages are recommended, in addition, the aridity of the climate and the chernozem pH values close to alkaline lead to a decrease in the herbicide decay rate, which increases the period of its active action in the soil.

The herbicide Lazurite (700 g/kg metribuzin) was used as an alternative (Chemical plant protection system 2). It is a systemic herbicide with a selective spectrum of action

Table 1. Field experiment scheme

No.	Variant	Fertilizers	Used preparations		
			Kind	Name	Application dose
1	Control	No fertilizers	–	–	–
2	Chemical plant protection system 1	No fertilizers	Herbicide	Gezagard	3.0l/ha
			Insecticide	Bi-58 New	1.0 l/ha
3	Chemical plant protection system 2	No fertilizers	Fungicide	Sinclair	0.6 l/t
			Herbicide	Lazurit	1.0kg/ha
			Fungicide	Optimo	0.5 l/ha
			Insecticide	Ampligo	0.2 l/ha
4	Biological plant protection system	No fertilizers	Fungicide	Planriz	0.3 l/t
			Biopreparation	Geostim	1.0l/ha
5	Control	$N_{40}P_{40}K_{40}$	–	–	–
6	Chemical plant protection system 1	$N_{40}P_{40}K_{40}$	Herbicide	Gezagard	3.0l/ha
			Insecticide	Bi-58 New	1.0 l/ha
7	Chemical plant protection system 2	$N_{40}P_{40}K_{40}$	Fungicide	Sinclair	0.6 l/t
			Herbicide	Lazurit	1.0 kg/ha
			Fungicide	Optimo	0.5 l/ha
			Insecticide	Ampligo	0.2 l l/t ha
8	Biological plant protection system	$N_{40}P_{40}K_{40}$	Fungicide	Planriz	0.3 l/t
			Biopreparation	Geostim	1.0l/ha

against monocotyledonous and dicotyledonous weeds, belongs to the class of triazinones. Like Gezagard, Lazurite is recommended to be used only before germination.

Metribuzin decomposes in the soil within three months and can have a toxic effect on the cultivated cropwhich manifests itself against the background of plant stress due to insufficient or excessive moisture in the soil, damage by diseases or pests.

Sinclair, a concentrated fungicidal seed dressing, has been used to control a wide range of seed and soil borne diseases. During the growing season, contact fungicide Optimo (200 g/l pyraclostrobin) was used. Pyraclostrobin is destroyed in the presence of sunlight, but when it enters the soil, it remains stable for up to 10 years. With regular use, especially when the recommended dosages are exceeded, it accumulates in the soil and can not only cause a toxic effect on pathogenic microflora, but also reduce the number of all groups of microorganisms in the surface layer of the soil.

Protection of crops from pests is an important condition for obtaining high yields of leguminous crops. Insecticides Bi-58 New (400 g/l dimethoate) and Ampligo (50 g/l lambda-cyhalothrin + 100 g/l chlorantraniliprole) were used to control such pests as cotton bollworm, pea aphid, pea codling moth, and pea weevil.

Planriz is a contact biological fungicide containing Pseudomonas fluorescens strain AP 33. This preparation was used in a variant with a biological plant protection system for pre-sowing seed treatment.

The microbiological preparation Geostim is used for biological control and protection against a wide range of diseases caused by fungi. Associative microorganisms that are part of the preparation have a stimulating effect on plants, forming symbiotic bonds with cultivated plants. When vegetative plants are treated with it, biochemical processes are enhanced, and the activity of plant enzymes is increased.

The enzymatic activity of the soil was determined by the activity of enzymes of the hydrolase class (invertase, phosphatase, urease) and the class of oxidoreductases (catalase). The analysis was carried out according to generally accepted methods in dry soil samples, cleaned of plant residues and sieved through a sieve with holes of 1 mm in diameter [10]. Catalase activity was determined by the gas volumetric method, by measuring the volume of released oxygen formed during the interaction of hydrogen peroxide with soil; phosphatase activity - by the method of hydrolysis of sodium phenolphthalein phosphate; invertase activity - by a photocolorimetric method based on the quantitative accounting of reducing sugars formed during the breakdown of sucrose under the enzyme action (according to Bertrand). Urease activity was determined by the method of A. Sh. Galstyan with the determination of ammonia by photometry of a colored solution of an indophenol compound formed by the interaction of ammonium with salicylate and sodium hypochlorite in an alkaline medium [11].

3 Results and Discussion

Soil enzymes are involved in the most important processes that form the basis of soil metabolism - the transformation of entering the soil carbohydrates, nitrogen and organophosphorus compounds and the biogenesis of humus. Thus, to a certain extent, enzymes determine the direction and degree of expression of soil-forming processes, the level of fertility, control the soil evolution, and characterize the degree of agroecosystems disturbance arising under the influence of natural and anthropogenic factors.

Catalase belongs to the class of oxidoreductases that catalyze redox reactions in the soil. Hydrogen peroxide, which is toxic to plants, is continuously formed in the soil as a result of biochemical reactions in the oxidation of organic compounds, the metabolism of aerobic microorganisms, and in the process of plant respiration. An important role of catalase is the destruction of peroxide to molecular oxygen and water [1]. The activity of catalase largely depends on soil moisture, pH and its temperature regime. Excessive or insufficient soil moisture, as well as a decrease in temperature, adversely affect the rate of enzymatic reactions. The optimal pH values for catalase are in the range of 7.2–8.3.

The average catalase activity in the upper layer (0–20 cm) of ordinary chernozem under chickpea crops varied in all variants within the range of 9.6–12.3 ml O_2 per 1 g of soil per 1 min (Table 2). According to the scale of D.G. Zvyagintsev for assessing the degree of soil enrichment with enzymes, chernozem in most cases falls into the category of rich (10–30 O_2 cm^3/g per 1 min) in terms of the content of catalase in it.

The coefficient of variation of catalase before treatment with drugs was 4.11% on the variants without fertilizer and 3.75% on the fertilized background, which indicates a slight difference in data scatter. After the use of plant protection products, the coefficient of variation of the activity index between the variants slightly increases (6.37% and 7.39%, respectively).

The influence of plant protection agents on the enzymatic activity of soils has been repeatedly discussed in the literature [9, 12, 13]. The data are contradictory, both stimulating and inhibitory effects of various drugs on enzymes are noted. This is due both to the variety of preparations used, and to the complexity of the processes occurring in the "soil-microorganisms-plants" system, as well as to the diversity of the soils themselves and the natural and climatic conditions in which they are located.

Table 2. Dynamics of catalase activity (cm^3 O_2 per 1 g of soil per 1 min) depending on the background of fertilizers and plant protection system

Variant	Catalase activity, cm^3 O_2 per 1 g of soil per 1 min		Change in values (%)	Change compared to the control (%)
	Before treatment	After treatment		
1	11.6 ± 1.2	10.0 ± 0.4	−13.8	–
2	11.4 ± 1.6	10.3 ± 1.0	−9.7	+3.0
3	12.3 ± 0.9	10.3 ± 1.1	−16.3	+3.0
4	10.9 ± 1.5	10.3 ± 1.5	−5.5	+3.0
5	11.9 ± 0.5	9.9 ± 0.2	−16.8	–
6	11.9 ± 0.3	10.0 ± 0.7	−16.0	+1.0
7	12.1 ± 1.6	9.6 ± 0.7	−20.7	−3.0
8	11.2 ± 1.4	11.3 ± 0.8	+0.9	+12.4

In our studies, after treatment with chemical plant protection products of both the first and second groups, a tendency for a decrease in catalase activity in the soil was revealed. It is most clearly expressed in the variants on the background of fertilizers. The statistically significant difference between the indicators of catalase activity before and after treatment on variants without fertilizers was 1.1 and 2.0, respectively, and on variants with fertilizers - 1.9 and 2.5. The small positive difference when compared with the control after drug treatment is not statistically significant.

When chickpeas are treated with biological protection preparations, the change in catalase activity is statistically insignificant both in the variants without fertilizers and in the presence of fertilizers.

Hydrolases are represented in soils by fairly large groups of enzymes that split peptide, acid anhydrite, ester, glycosidic, and some other bonds in high molecular weight organic compounds, releasing nitrogen and phosphorus available to plants and microorganisms [6, 7]. In the present work, we studied the activity of invertase, phosphatase, and urease from enzymes of the hydrolase class.

Invertase is involved in the biochemical transformation of carbohydrates, splitting the glycosidic bonds of sucrose to monomers. The highest indicators of invertase activity are typical for the upper part of the humus profile. Invertase closely correlates with the humus content and decreases down the soil profile.

According to invertase activity, the studied ordinary chernozem belongs to the category of moderately enriched according to the scale of D. G. Zvyagintsev to assess the degree of soil enrichment with enzymes. The average indicators of invertase activity varied in all variants within the range of 30.4–36.8 mg of glucose per 1 g of soil for 24 h (Table 3).

The coefficient of variation of invertase before treatment with drugs was 4.89% on the variants without fertilizer and 2.84%on the fertilizer background, which indicates a slight difference between the data. After treatment, the coefficient of variation increased and amounted to 6.75 and 6.23%, respectively.

Analysis of the data showed that after the application of various plant protection products, a decrease in invertase activity was noted in all variants. The largest statistically significant decrease in indicators was observed in variant 2 with Gezagard - by 7.2% without the use of fertilizers and by 15.6% against the background of fertilizers. At the same time, a decrease in invertase activity was also noted in the control, where no drugs were added. This can probably be associated with certain seasonal phenomena (decrease in soil moisture and increase in temperature).

Table 3. Dynamics of invertase activity (mg glucose per 1 g of soil per 24 h) depending on the background of fertilizers and plant protection system

Variant	Invertase activity, mg glucose per 1 g per 24 h		Change in values (%)	Change compared to the control (%)
	Before treatment	After treatment		
1	36.0 ± 1.7	34.2 ± 1.1	−5.0	–
2	36.1 ± 1.1	33.5 ± 1.4	−7.2	−2.0
3	31.8 ± 1.6	30.6 ± 1.0	−3.8	−10.5
4	36.8 ± 0.8	35.0 ± 1.6	−4.9	+2.3
5	36.0 ± 1.1	34.7 ± 1.0	−3.6	–
6	36.0 ± 1.0	30.4 ± 0.3	−15.6	−12.3
7	36.0 ± 1.2	33.5 ± 0.7	−6.9	−3.4
8	34.4 ± 1.8	33.0 ± 0.9	−4.1	−4.9

Phosphatase catalyzes the hydrolysis of organophosphorus compounds by phosphorus-ether bonds. Thus, it is responsible for the mineralization of organic phosphorus, and the activity of phosphatase characterizes the intensity of biochemical processes associated with it. By releasing phosphoric acid from organic compounds entering the soil with plant residues, this enzyme plays an important role in providing plants with available phosphorus. With its deficiency in the soil, additional release of enzymes by microorganisms and plants occurs, which leads to an increase in phosphatase activity.

The converse statement is also true: with the accumulation of a large amount of mobile phosphorus, the activity of phosphatase decreases [1].

According to the enrichment of the phosphatase enzyme, the studied ordinary chernozems belong to the middle category, the average indicators of phosphatase activity for all variants varied within 2.3–4.3 mg P_2O_5 per 10 g per 1 h (Table 4). The coefficient of variation in the values of phosphatase activity before treatment with drugs was 12.66% for the variants without fertilizer and 10.52% for the average background of fertilizers, which indicates an average degree of data scatter. After treatment, the variability of the trait remained at the same level and amounted to 10.52% and 11.82%, respectively, on different backgrounds.

Table 4. Dynamics of phosphatase activity (mg P_2O_5 per 10 g of soil per 1 h) depending on the background of fertilizers and plant protection system

Variant	Phosphatase activity, mg P_2O_5 per 10 g per 1 h		Change in values (%)	Change compared to the control (%)
	Before treatment	After treatment		
1	2.7 ± 0.9	3.8 ± 1.1	+28.9	–
2	2.9 ± 0.6	4.3 ± 0.2	+32.6	+9.3
3	2.5 ± 0.7	4.0 ± 0.1	+37.5	+5.0
4	3.5 ± 0.5	3.5 ± 0.7	0.0	−11.4
5	2.7 ± 0.4	3.5 ± 0.1	+22.9	–
6	2.3 ± 0.4	3.5 ± 0.2	+34.3	0.0
7	2.5 ± 0.2	3.4 ± 0.8	+26.5	−2.9
8	2.8 ± 0.2	2.8 ± 0.2	0.0	−25.0

Analysis of the data obtained showed that the treatment of plants with chemical protection agents increased the activity of phosphatase in the soil. An increase in phosphatase activity is also observed in the control. This may indicate the active absorption of phosphorus by plants, which causes a decrease in the amount of available phosphorus in the soil and, accordingly, an increase in its phosphatase activity. A statistically significant increase in phosphatase activity was noted both in the variants without fertilizer and in the presence of fertilizer.

In variants with a biological plant protection system, phosphatase activity remains approximately at the same level before and after the application of plant protection products, which indicates an established equilibrium in the "soil – plant" system.

Urease catalyzes the hydrolysis of carbamide (urea) by splitting the bond between nitrogen and carbon (CO–NH). In the soils of agrocenoses, carbamide is applied in a significant amount in the form of nitrogen fertilizer, and also enters the soil with manure and plant residues. In the soil itself, urea is formed as an intermediate product in the process of transformation of nitrogenous organic compounds – proteins and nucleic acids.

The reaction of hydrolysis of urea can be considered as a process of ecological mineralization of organic nitrogen-containing compounds, as a result of which the water-soluble substrate (urea) is transformed into volatile products - ammonia and carbon dioxide. As a result, urea is converted into an easily accessible ammonium salt, which serves as a direct source of nitrogen nutrition for autotrophic organisms, including higher plants [1].

Urease in the soil is associated with the organomineral complex and is highly resistant to inhibitory factors. The optimal pH value for urease is 6.5–7. In an acidic and strongly alkaline environment, urease activity decreases. The highest urease activity is characteristic of the humus horizon and decreases down the profile. However, as our studies have shown, the distribution of urease along the chernozem profile in the agrocenosis is irregular, with a maximum accumulation in the 20–60 cm layer and a reduced content in the arable horizon. This distribution is explained by the high anthropogenic impact on the arable horizon of the soil.

The average values of urease activity in the studied chernozems under chickpea crops varied from 4.5 to 8.1 mg of $N–NH_3$ per 10 g of soil in 24 h (Table 5) and according to the D.G. Zvyagintsev soils fall into the category of poor ones. The coefficient of variation of urease activity indicators before treatment with drugs on variants without fertilizer shows an insignificant scatter of data (7.32%), and on a fertilized background - an average degree of data scatter (12.13%). After treatment with plant protection products, the coefficient of variation was 7.12–7.74%.

Table 5. Dynamics of urease activity (mg $N-NH_3$ per 10 g of soil in 24 h) depending on the background of fertilizers and plant protection system

Variant	Urease activity, mg of N-NH3 per 10 g in 24 h		Change in values (%)	Change compared to the control (%)
	Before treatment	After treatment		
1	7.2 ± 0.8	5.9 ± 0.8	−18.1	–
2	7.2 ± 1.2	5.9 ± 0.6	−18.1	0.0
3	7.9 ± 0.7	6.7 ± 0.4	−15.2	+3.7
4	7.4 ± 0.9	6.0 ± 0.2	−18.9	+1.7
5	7.1 ± 0.5	5.5 ± 0.6	−22.5	–
6	6.0 ± 0.2	4.5 ± 0.3	−25.0	−18.2
7	8.1 ± 0.4	5.7 ± 0.7	−29.6	+3.5
8	7.1 ± 0.8	5.8 ± 0.6	−18.3	+5.2

There is a trend towards a decrease in urease activity after treatment with pesticides, however, a statistically significant decrease occurs in the control. Compared with the control, almost all variants showed a slight statistically insignificant increase in urease activity. Thus, despite the decrease in activity, we cannot assert that it occurred due to pesticide treatments, and not due to natural seasonal phenomena. The exception is variant 6 with chemical protection 1 on a fertilized background, where after treatment with pesticides a statistically significant decrease in urease activity was observed both in comparison with the values before treatment and in comparison with the control.

4 Conclusions

Thus, according to the D.G. Zvyagintsev enzyme enrichment scale, the investigated ordinary chernozem belongs to rich in catalase, moderately enriched in invertase and phosphatase, and poor in urease. The scatter of data between the variants before treatment with drugs is insignificant for catalase, invertase and urease (2.84–7.32%) and average for phosphatase 10.52–12.66%).

Treatment with chemical plant protection products of both the first and second groups has a different effect on various enzymes, reducing the activity of catalase, invertase and urease and stimulating the activity of phosphatase. After treatment with drugs, the variability of catalase and invertase activity between the variants increased to 7.39 and 6.75%, respectively.

When treated with biological plant protection products, catalase activity is stimulated and invertase and urease activity is inhibited. The treatment did not have a significant effect on the activity of phosphatase, it remained at the same level.

References

1. Rao, M.A., Scelza, R., Acevedo, F., et al.: Enzymes as useful tools for environmental purposes. Chemosphere **107**, 145–162 (2014)
2. Du, Z., Zhu, Y., Zhu, L., et al.: Effects of the herbicide mesotrione on soil enzyme activity and microbial communities. Ecotoxicol. Environ. Saf. **164**, 571–578 (2018)
3. Woźniak, A.: Chemical properties and enzyme activity of soil as affected by tillage system and previous crop. Agriculture **9**(12), 262 (2019)
4. Borase, D.N., Nath, C.P., Hazra, K.K., et al.: Long-term impact of diversified crop rotations and nutrient management practices on soil microbial functions and soil enzymes activity. Ecol. Ind. **114**, 106322 (2020)
5. Kuscu, I.S.K., Yigit, N., Savaci, G., et al.: Relationship between enzyme activity (Urease-catalase) and nutrient element in soil use. Pol. J. Environ. Stud. **27**(5), 2107–2112 (2018). https://doi.org/10.15244/pjoes/78475
6. Borisov, A.V., Demkina, T.S., Kashirskaya, N.N., et al.: Changes in the past soil-forming conditions and human activity in soil biological memory: microbial and enzyme components. Eurasian Soil Sci. **54**(7), 1078–1088 (2021). https://doi.org/10.1134/S1064229321070024
7. Li, Y., Zhang, L., Fang, S., Tian, Y., Guo, J.: Variation of soil enzyme activity and microbial biomass in poplar plantations of different genotypes and stem spacings. J. For. Res. **29**(4), 963–972 (2017). https://doi.org/10.1007/s11676-017-0524-2
8. World Reference Base for Soil Resources 2014. International soil classification system for naming soils and creating legends for soil maps. Update 2015. World Soil Resources Reports 106, p. 192. FAOUN, Rome
9. Bezuglova, O.S., Gorovtsov, A.V., Polienko, E.A., et al.: Effect of humic preparation on winter wheat productivity and rhizosphere microbial community under herbicide-induced stress. J. Soils Sediments **19**, 2665–2675 (2019). https://doi.org/10.1007/s11368-018-02240-z
10. Khaziev, F.K.: Methods of Soil Enzymology, p. 252. Nauka, Moscow (2005)

11. Naimi, O.I.: On the method for determining the activity of urease in the soil. In: High Technologies and Innovations in Science: Proceedings of the International Scientific Conference, pp. 17–20. GNII National Development, St. Petersburg (2019)
12. Liu, J.-H., Wang, Q., Sun, S.-J., et al.: Effect of fulvic acid fertilizer on microbial diversity and enzyme activity in wheat rhizosphere soil. J. Plant Nutr. Fertilizers **27**(4), 1808–1816 (2019)
13. Schofield, E.J., Brooker, R.W., Paterson, E., et al.: Plant-plant competition influences temporal dynamism of soil microbial enzyme activity. Soil Biol. Biochem. **139**, 107615 (2019)